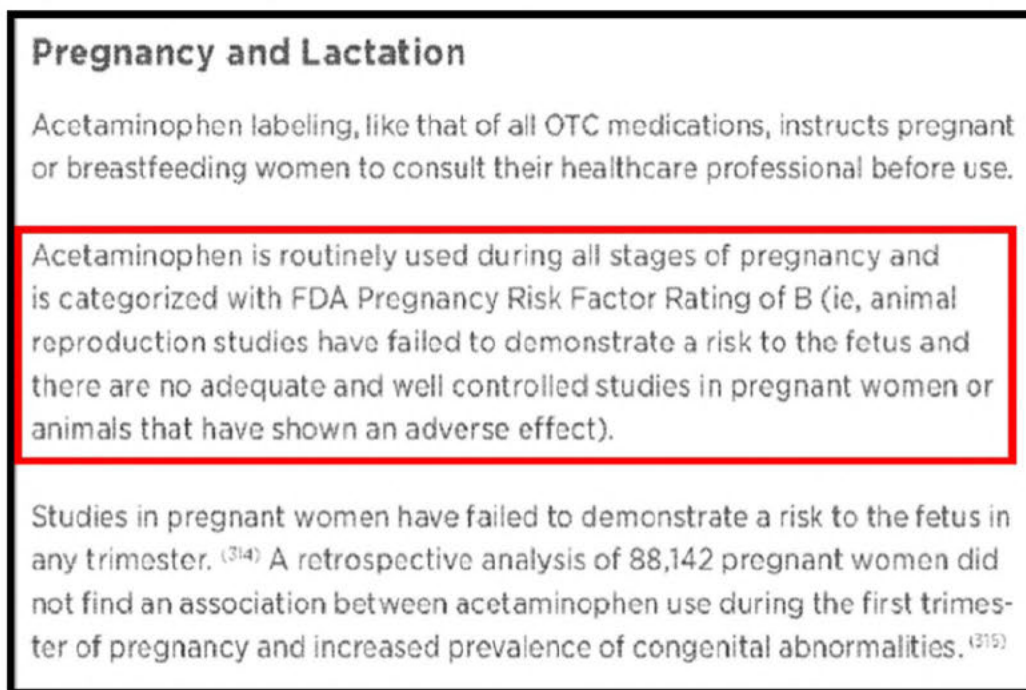


EXHIBIT 6h

Of the 52 studies that described experiments designed to test the safety of APAP in infants or children, most examined only liver toxicity. Of the five studies that monitored outcomes 28 or more days after APAP exposure, none of them monitored neuropsychiatric function (see Fig. 2 in Candeias-Hernandez et al.) In the absence of adequate and well-controlled studies in pregnant women coupled with the animal studies showing adverse effects on the fetus as detailed in the Ofirmev and Ultracet labels, APAP would not meet the criteria for pregnancy category B.

I reviewed JJCI's 2015 Tylenol Professional Product Information⁴¹⁴, which was available on their website TylenolProfessional.com and states:



In the field of teratology, which seeks to minimize the risk of birth defects through education, research, and public health initiatives, the FDA pregnancy categories were a tool by which pregnant women and their healthcare providers could readily evaluate the potential teratogenic risks associated with a drug in deciding whether the potential risks outweighed the benefits. The lack of consistency in the teratogenic risk information presented for APAP products undermines the goals of teratology and prohibits pregnant women from accessing the information they need to make informed decisions.

⁴¹⁴ APAP-JJCI-0000180610; According to the deposition testimony of [REDACTED], the pregnancy section of TylenolProfessional.com was last updated in 2015, and is no longer available on the website. [REDACTED] Dep. 297:16-315:2,

G. AOP and WoE Based Conclusion from Animal Studies

Several conclusions can be drawn from these animal studies. These conclusions are presented based on applying the weight of evidence (WoE) to the Oxidative Stress Adverse Outcome Pathway (AOP) that identifies APAP as a stressor.

The NTP (National Toxicology Program) has published guidelines for rating the strength of the evidence for drawing conclusions from a study.⁴¹⁵ The guidelines define five levels of evidence:

“Levels of Evidence for Evaluating Developmental System Toxicity

- **Clear evidence of developmental toxicity** is demonstrated by data that indicate a dose-related effect on one or more of its four elements (embryo-fetal death, structural malformations, growth retardation or functional deficits) that is not secondary to overt maternal toxicity.
- **Some evidence of developmental toxicity** is demonstrated by dose-related effects on one or more of its four elements (embryo-fetal death, structural malformations, growth retardation or functional deficits), but there are greater uncertainties or weaker relationships with regard to dose, severity, magnitude, incidence, persistence, and/or decreased concordance among affected end points.
- **Equivocal evidence of developmental toxicity** is demonstrated by marginal or discordant effects on developmental parameters that may or may not be related to the test article.
- **No evidence of developmental toxicity** is demonstrated by data from a study with appropriate experimental design and conduct that are interpreted as showing no biologically relevant effects on developmental parameters that are related to the test article.
- **Inadequate study of developmental toxicity** is demonstrated by a study that, because of major design or performance flaws, cannot be used to determine the occurrence of developmental toxicity.”

Conclusion 1: Perinatal APAP exposure via NAPQI is reported to decrease GSH and increase adduct formation.

Exposing mice and rats during development to acetaminophen at human equivalent doses causes significantly reduced glutathione levels and significantly increased oxidative stress, including in the brain. Multiple studies in both mice and rats demonstrate these effects. There is “clear evidence” supporting dose-responsive, physical-chemical interactions between NAPQI and GSH and protein-thiols. These studies and supporting data are peer-reviewed, replicated, and include high quality regulatory studies. This AOP interaction is supported based on the WoE.

Conclusion 2: Perinatal APAP exposure via NAPQI is reported to cause oxidative stress.

Exposing mice and rats during development to acetaminophen at human equivalent doses causes significantly increased oxidative stress. Oxidative stress is measured and supported by standardized assays, including TBARS. There is “clear evidence” supporting dose-responsive production of oxidative stress by NAPQI. These studies and supporting data are peer-reviewed, replicated, and include high quality regulatory studies. This AOP interaction is supported based on the WoE.

Conclusion 3: Perinatal APAP exposure via NAPQI is reported to result in neurotoxicity.

⁴¹⁵ NTP- Explanation of Levels of Evidence for Developmental Toxicity. <http://ntp.niehs.nih.gov/go/10003>. Paul M. Foster.

Exposing mice and rats during development to acetaminophen at human equivalent doses during neural development causes significant neurodevelopmental impacts to mice and rats. A total of 22 studies (8 mice and 14 rats) investigated whether early-life exposure to acetaminophen caused biological changes in the brain. Animal studies show “clear evidence” of developmental toxicity, consistent with regulatory guidelines. The evidence of developmental toxicity is strengthened because multiple studies show increases in severity and/or prevalence as a function of dose (dose responsive toxicity was observed in multiple studies), effects seen in litter-based analyses provide stronger evidence (multiple litters impacted across multiple studies), and concordant effects (oxidative damage causing DNA oxidation, reproductive toxicity, and neurodevelopment toxicity). Finally, consistent with “clear evidence” of developmental toxicity, these end points are reported as statistically and biologically significant across multiple litters tested and multiple studies.⁴¹⁶

The following animal studies provide “clear evidence” of developmental toxicity, with oxidative stress and oxidative DNA damage, consistent with developmental neurotoxicity due to perinatal APAP exposure.

Mice.

Animal Study	Year	Evidence
Ruepp	2002	decreased glutathione, loss of ATP production, and enlargement and fusion of mitochondria
De Silva	2012	systemic depletion of glutathione and resulting brain damage
Markovic	2013	decreased glutathione and DNA damage consistent with oxidative stress
Viberg	2014	changes in the levels of brain derived neurotropic factor
Hay-Schmidt	2017	decrease in the number of neurons in a portion of the hypothalamus
Philippot	2018	reduction in cerebral cortical fatty acid amide hydroxylase (FAAH), and reduction of transcript levels of hippocampal synaptophysin (Syp) and tropomyosin receptor kinase B
Philippot	2022	acute oxidative stress in the hippocampus
Baker	2023	altered prefrontal cortex gene expression related to various molecular pathways

Rats.

Animal Study	Year	Evidence
Harris	1989	abnormal closure of the anterior neuropore
Dean	2012	increased spinolin and dendritic growth
Blecharz-Klin	2013	levels of monoamines and metabolites
Blecharz-Klin	2014	decreased amino acids in the striatum (glutamine, glutamic acid, taurine, alanine, aspartic acid) and hypothalamus (glycine); increased levels of γ -aminobutyric acid (GABA) in the prefrontal cortex
Blecharz-Klin	2015a	increased aspartic and glutamic acid in the spinal cord

⁴¹⁶ NTP, Explanation of Levels of Evidence for Developmental Toxicity (<http://ntp.niehs.nih.gov/go/10003>)

Blecharz-Klin	2015b	altered dopaminergic system in the medulla oblongata
Lichtensteiger	2015	altered gene expression in the hypothalamus
Blecharz-Klin	2017	amino acid levels in the hippocampus
Blecharz-Klin	2018	decreased BDNF in the striatum
Blecharz-Klin	2019	altered levels of monoamines, metabolites, amino acids, dopamine, and noradrenaline in the hypothalamus
Saeedan	2018	increase increased oxidative markers (TBARS) and inflammatory markers
Klein	2020	reduced glutathione and brain derived neurotrophic factor
Koehn	2020	increased cytokines and immune-response genes, and reduced acute phase plasma proteins
Rigobello	2021	decreased glutathione

Perinatal APAP exposure is reported to impair learning or social behavior.

Exposing mice and rats during development to APAP at human equivalent doses during neural development causes significantly altered learning, locomotor, and social behavior consistent with ASD and ADHD. A total of 17 studies (10 in mice and 7 in rats) investigated whether early-life exposure to acetaminophen caused behavioral changes consistent with ASD and ADHD. Of these, 15 of the 17 showed that APAP caused impaired learning and altered behaviors.⁴¹⁷ Accordingly, these studies provide both “clear evidence” and “some evidence” of developmental toxicity. The other 2 studies used low dosages (Blecharz-Klin 2017) or multifactorial non-DART experiments (Zhao 2017) that showed “no evidence” in the WoE for impaired learning or social behavior. The “no evidence” is proposed to be a result of the sub-therapeutic dose in the former study. The studies by Zhao include multiple variables that make interpretation a circular paradox, often termed begging the question, this includes LPS causing inflammation and fevers, APAP causing toxicity, APAP reducing inflammation or fever reduction, reported LPS mitigation of APAP toxicity, and reciprocated APAP mediation of LPS toxicity. As both agents potentiate toxicity but mitigate the toxicity of each other, “no evidence” is one expected outcome. Consistent with neurobehavioral study guidelines, these animal studies show “clear evidence” that perinatal APAP exposure results in learning deficits and impaired social behavior.

Mice

Animal Study	Year	Evidence
Ishida	2017	clear evidence of learning deficits
Gould	2012	clear evidence of repetitive behavior, social interactions
Viberg	2014	clear evidence of ambulation and learning deficits

⁴¹⁷ In addition, Diaz-Estrada 2021 concluded that acetaminophen exposure during neurodevelopment caused males to have increased homosexual partner preference, and Hay-Schmidt 2017 found that acetaminophen exposure resulted in altered sexual behavior.

Saad	2016	clear evidence of locomotor activity, ambulation, fine repetitive movements
Zhao	2017	Inadequate study of developmental toxicity. The 2017 study was conducted in adult mice treated with LPS or APAP, and APAP 100mg/Kg did not differ from control in behavioral testing in the absence of LPS.
Philippot	2017	clear evidence of deficit in habituation capability
Philippot	2018	clear evidence of altered spontaneous behavior and habituation capability in a novel home cage
Philippot	2022	clear evidence of impaired habituation capability, reduced memory and learning ability, and decreased cognitive flexibility
Harshaw	2022	clear evidence of impaired social-emotional and repetitive behaviors
Baker	2023	clear evidence of impaired social behavior

Rats

Animal Study	Year	Evidence
Dean	2012	clear evidence of social interaction and sensory threshold in males
Blecharz-Klin	2017	equivocal evidence, alterations in cognitive functions, improved spatial memory and search strategy at sub-therapeutic doses (5mg/Kg), but no significant differences at increased dosing (15mg/Kg).
Blecharz-Klin	2018	clear evidence of a lower frequency of social interactions, greater pinning behavior, and rats from the 15mg/kg group exhibited a greater interest in objects in the novel object recognition tests.
Saeedan	2018	some evidence of impaired eye opening, locomotor activity, olfactory discrimination, and on entries in the elevated plus maze.
Klein	2020	clear evidence of impaired nest seeking behavior and decreased rostral grooming in the elevated plus maze.
Rigobello	2021	clear evidence of augmented stereotyped behavior and ambulation, reduction in exploratory behavior.
Suda	2021	clear evidence of increased asocial rearing behavior

Conclusion 5: Perinatal APAP exposure is reported to cause developmental or reproductive toxicity.

Acetaminophen exposure during reproductive and developmental toxicity testing caused both mice and rats to have alterations in reproductive and other tissues. These effects were consistent across multiple studies.

Conclusion 6: Perinatal APAP exposure shows dose, duration, and frequency dependent interactions.

The effects described above by exposing acetaminophen were dose and duration dependent. Exposure to greater doses and for longer durations increased the effects on brain tissues and behavior.

Conclusion 7: Perinatal APAP exposure is reported to cause specific and consistent toxicities.

The effects on brain tissues and behavior show a high degree of consistency, with over 90% of the studies supporting the hypothesis that APAP exposure during neurodevelopment at doses equivalent to therapeutic human doses causes significant changes in the brain and results in significant behavioral effects in later life.

5. Population (Human)

Reflective and supportive of the above-discussed animal studies, the scientific literature also supports increased risk of neurodevelopmental toxicity in humans associated with APAP exposure during pregnancy. Reported interactions between APAP and developmental toxicity endpoints include autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). The available epidemiological evidence is reviewed systematically and is organized into the following six categories:

- APAP with ASD
- APAP with ADHD
- APAP with impaired learning, cognitive, or social outcomes
- Meta-analyses of the Association of APAP with ASD or ADHD
- Other relevant reviews
- APAP and other birth outcomes

A. Studies Examining the Association of APAP with ASD

Avella-Garcia 2016

The study is a Spanish birth cohort study that investigated the association between prenatal exposure to acetaminophen and neurodevelopmental outcomes at 1 and 5 years of age.⁴¹⁸

Information used to determine exposure: The exposure of interest was acetaminophen use during pregnancy, which was assessed using two structured interviews that asked about medication use during pregnancy. Ever/never use and frequency of use (never, sporadic, persistent) were measured.

Outcome definition and determination: The main neurodevelopmental outcomes were assessed using the Childhood Autism Spectrum Test (CAST), Conner's Kiddie Continuous Performance Test (K-CPT), and ADHD-DSM-IV form list.

Control group: The control group consisted of children whose mothers did not report using APAP during pregnancy.

Study size: The study included 2644 mother-child pairs recruited during pregnancy. The proportion of liveborn participants evaluated at 1 and 5 years was 88.8% and 79.9%, respectively.

Confounding factors or biases and how or if they were controlled: The study controlled for several potential confounding factors, including social determinants and co-morbidities. Regression models were adjusted for these factors.

Limitations: Reported limitations included confounding by indication, despite adjustment, genetic confounding, residual confounding, self-reporting, and potential misclassification.

⁴¹⁸ Avella-Garcia et al. Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms. *Int J Epidemiol.* 2016 Dec 1;45(6):1987-1996. doi: 10.1093/ije/dyw115. PMID: 27353198.

Results: The study found that prenatal acetaminophen exposure was associated with a greater number of autism spectrum symptoms in males and showed adverse effects on attention-related outcomes for both genders. In this study, 43% of children evaluated at age 1 ($n = 2195$) and 41% of those assessed at age 5 ($n = 2001$) were exposed to APAP. Exposed children showed a higher risk of presenting hyperactivity/impulsivity symptoms than non-exposed children (incidence rate ratio (IRR) = 1.41, 95% CI 1.01–1.98), a greater risk of K-CPT commission errors (IRR = 1.10, 95% CI 1.03–1.17) and lower detectability scores ($\beta = 0.75$, 95% CI 0.13–0.02), than non-exposed children after full multivariate adjustment.

In conclusion, this study found that taking acetaminophen during pregnancy may lead to more symptoms of Autism Spectrum Condition (ASC) in male children. It also showed that prenatal exposure to this medication can affect attention function in 5-year-old children, but the effects may be different for boys and girls. The study also found a connection between acetaminophen exposure and hyperactivity/impulsivity behaviors in all children.

Bittker 2018

The study design was an internet-based survey.⁴¹⁹

Information used to determine exposure: Participants were parents living in the USA with at least one biological child between 3 and 12 years of age. Potential participants were informed about the survey via postings on social media, websites, and listservs and were offered an opportunity to participate in a raffle as well. Participants were also recruited through the Interactive Autism Network.

Study size: After exclusions, there remained 1,001 responses associated with children with ASD (cases) and 514 responses associated with children who do not have ASD (controls). The outcome definition was whether or not a child had ASD.

Confounding factors or biases and how or if they were controlled: The study adjusted for eight demographic variables when analyzing the association between the postnatal variables and ASD. Adjusted variables, e.g., education, as a scaled variable, race as binary 0 (White (non-Hispanic)) or 1 (other). Specific confounding factors or biases that were controlled for are not clearly indicated.

Limitations: Reported limitations include being an internet survey, population structure differences including gender distribution, mean age of child, maternal education, and, to a lesser extent, ethnicity.

Results: In this study, doses of postnatal acetaminophen, adjusted odds ratio (aOR) = 1.016, 95% CI 1.003–1.032), courses of postnatal antibiotics (aOR = 1.103, 95% CI 1.046–1.168), incidence of postnatal ear infection (aOR = 1.137, 95% CI 1.046–1.236), and decreased duration of breastfeeding (aOR 0.948, 95% CI 0.932–0.965) are all associated with ASD when adjusted for eight demographic variables. A weak association between oral vitamin D drop exposure and ASD was also found when adjusted for breastfeeding and demographics (aOR 1.025, 95% CI 0.995–1.056).

⁴¹⁹ Bittker et al. Acetaminophen, antibiotics, ear infection, breastfeeding, vitamin D drops, and autism: an epidemiological study. *Neuropsychiatr Dis Treat*. 2018 May 31;14:1399-1414. doi: 10.2147/NDT.S158811. PMID: 29910617; PMCID: PMC5987866.

In conclusion, this study adds to evidence that postnatal acetaminophen use, postnatal antibiotic use, incidence of ear infection, and early weaning are associated with an increased risk of ASD. It also finds that postnatal oral vitamin D drops are weakly associated with ASD when adjusted for breastfeeding and demographics.

Liew 2016c

The study is a prospective cohort study.⁴²⁰

Information used to determine exposure: The exposure of interest was acetaminophen use during pregnancy, which was assessed using three computer-assisted telephone interviews.

Outcome definition and determination: The outcome of interest was a diagnosis of ASD in the offspring, which was determined using records from the Danish hospital and psychiatric registries.

Control group: The control group consisted of children whose mothers did not report using acetaminophen during pregnancy.

Study size: The study included 64,322 children and mothers enrolled in the Danish National Birth Cohort (DNBC; 1996-2002) for an average of 12.7 years.

Confounding factors or biases and how or if they were controlled: The study controlled for several potential confounding factors, including maternal age, education, smoking during pregnancy, pre-pregnancy body mass index (BMI), parity, and hyperkinetic disorders. The authors used Cox proportional hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).

Limitations: Self-reported data from mothers, residual confounding by indication, or genetic factors.

Results: In this study, maternal use of APAP was associated with ASD by ever versus never use (aHR = 1.19, 95% CI 1.04-1.35), APAP use during all three trimesters (aHR = 1.39, 95% CI 1.14-1.70), and >20 weeks APAP use during pregnancy (aHR = 1.39, 95% CI 1.14-1.70). For infantile autism, often considered more severe, was associated with APAP use during all three trimesters (aHR = 1.49, 95% CI 1.07-2.07) and >20 weeks APAP use during pregnancy (aHR = 1.62, 95% CI 1.05-2.51). Inclusion of ASD or infantile autism with hyperkinetic symptoms was also reported to increase risk (Figure 32).

⁴²⁰ Liew et al. Maternal use of acetaminophen during pregnancy and risk of autism spectrum disorders in childhood: A Danish national birth cohort study. *Autism Res.* 2016 Sep;9(9):951-8. doi: 10.1002/aur.1591. Epub 2015 Dec 21. PMID: 26688372.

	Autism spectrum disorders				Infantile autism			
	Hyperkinetic –		Hyperkinetic +		Hyperkinetic –		Hyperkinetic +	
	No. cases	Adjusted HR ^a (95% CI)	No. cases	Adjusted HR ^a (95% CI)	No. cases	Adjusted HR ^a (95% CI)	No. cases	Adjusted HR ^a (95% CI)
Acetaminophen use during pregnancy								
Never used	295	1.00 (ref)	106	1.00 (ref)	110	1.00 (ref)	29	1.00 (ref)
Ever used	412	1.07 (0.92–1.24)	214	1.51 (1.19–1.92)	147	0.98 (0.77–1.26)	59	1.55 (0.98–2.45)
All three trimesters	87	1.25 (0.98–1.60)	46	1.77 (1.24–2.53)	33	1.23 (0.83–1.83)	17	2.45 (1.32–4.53)
Weeks of acetaminophen use throughout pregnancy								
0 weeks	295	1.00 (ref)	106	1.00 (ref)	110	1.00 (ref)	29	1.00 (ref)
1–5 week	203	1.06 (0.88–1.27)	105	1.51 (1.14–2.00)	73	1.00 (0.74–1.34)	25	1.32 (0.75–2.31)
6–20 weeks	50	0.99 (0.73–1.34)	31	1.62 (1.08–2.45)	15	0.78 (0.46–1.34)	8	1.55 (0.68–3.50)
>20 weeks	44	1.42 (1.02–1.97)	22	1.89 (1.19–3.02)	17	1.45 (0.86–2.43)	7	2.25 (1.00–5.07)
		P-trend = 0.074		P-trend = 0.019		P-trend = 0.233		P-trend = 0.071

Figure 32. Hazard Ratios for ASD and Infantile Autism with Hyperkinetic Symptoms. Adjusted child's sex, birth year, maternal age at birth, parity, socioeconomic status, maternal smoking and alcohol drinking during pregnancy, maternal pre-pregnancy body mass index, folic acid intake during pregnancy, mother's psychiatric illnesses, maternal diseases in muscles/joints, fever, or infection/inflammation during pregnancy, maternal use of ibuprofen and aspirin during pregnancy. (Liew et al 2016c)

Overall, the study found that prenatal use of acetaminophen was associated with an increased risk of ASD accompanied by hyperkinetic symptoms but not with other ASD cases. Longer duration of use (i.e., use for >20 weeks in gestation) increased the risk of ASD or infantile autism with hyperkinetic symptoms almost twofold.

Saunders 2019 (~)

The study is a case-control study that investigated the association between prenatal exposures and the risk of autism spectrum disorder (ASD) in children.⁴²¹

Information used to determine exposure: The exposure of interest was prenatal exposures, which were assessed using a questionnaire that asked about medication use, smoking, alcohol consumption, and other environmental exposures during pregnancy.

Outcome definition and determination: The outcome of interest was a diagnosis of ASD in children, which was determined using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria.

Control group: The control group consisted of children without ASD who were matched to cases by age and sex.

Study size: The study included 215 mothers of children (107 with ASD and 108 without ASD).

Confounding factors or biases and how or if they were controlled: The study controlled for several potential confounding factors, including maternal age, education, income, and pre-pregnancy body mass index (BMI). The authors used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

Limitations: Limitations included being a self-reported questionnaire from mothers, which may have reduced accuracy due to poor recall or biased recall. For example, none of the participants indicated

⁴²¹ Saunders et al. A Comparison of Prenatal Exposures in Children with and without a Diagnosis of Autism Spectrum Disorder. *Cureus*. 2019 Jul 24;11(7):e5223. doi: 10.7759/cureus.5223. PMID: 31565625; PMCID: PMC6758968.

alcohol consumption, although some participants admitted to using illicit drugs. Another limitation is that the study had a large number of variables and smaller sample sizes per group, which made it difficult to assess statistically the number of cigarettes smoked, types of medications used, and the formulation of the prenatal vitamin. Additionally, socioeconomic status (SES) was not controlled for in the study.

Results: The authors reported that 46.7% of mothers of children with ASD and 48.3% of mothers of children without ASD reported using APAP during pregnancy. Significant associations (Chi-square test) between multiple variables, including sibling with ASD, family member with ASD, medication use, and cigarette use. Significant odds ratios were reported for ASD family (OR 2.72, 95% CI 1.29-5.73), use of other medications (OR 2.29, 95% CI 1.21-4.36), and smoking (OR 2.56, 95% CI 1.91-5.49).

Overall, the study found that there was no statistically significant association found between APAP use during pregnancy and a later diagnosis of ASD in children. The lack of adjustment by SES was not controlled for in the study. Other studies have reported that high SES is associated with APAP use, improved developmental indices and inversely with smoking. In the absence of controlling for SES, the authors indicate, “Without controlling for these missing pieces, the current model cannot fully account for all variables in the complex diagnosis of ASD.”

Ji 2020

This is a prospective cohort study design using samples selected from the Boston Birth Cohort.⁴²²

Information used to determine exposure: Information about acetaminophen exposure was based on cord plasma biomarkers of fetal exposure to acetaminophen.

Outcome definition and determination: The outcomes of interest were childhood attention-deficit/hyperactivity disorder and autism spectrum disorder. Diagnoses were obtained from electronic medical record review.

Control group: The control group consisted of children without attention-deficit/hyperactivity disorder or autism spectrum disorder diagnosis.

Study size: The study included a total of 996 mother-infant dyads.

Confounding factors or biases and how or if they were controlled: Potential covariates such as maternal age, race/ethnicity, education, marital status, pre-pregnancy body mass index, smoking during pregnancy, alcohol use during pregnancy, and child sex were included in the statistical model.

Limitations: The study excluded confounding by indication but was unable to exclude the potential residual confounders of genetic and environmental factors.

Results: The highest concentrations of APAP in cord blood were associated with ASD (aOR = 2.88, 95% CI 1.80-4.66), ADHD (aOR = 3.72, 95% CI 1.70-8.55), and ASD and ADHD (aOR = 3.38, 95% CI 1.25-

⁴²² Ji et al. Association of Cord Plasma Biomarkers of In Utero Acetaminophen Exposure With Risk of Attention-Deficit/Hyperactivity Disorder and Autism Spectrum Disorder in Childhood. *JAMA Psychiatry*. 2020 Feb 1;77(2):180-189. doi: 10.1001/jamapsychiatry.2019.3259. PMID: 31664451; PMCID: PMC6822099.

9.85). Other developmental disorders were not associated with APAP concentrations (aOR = 0.86, 95% CI 0.56-1.31).

Overall, the study reported that cord plasma biomarkers of fetal exposure to acetaminophen were associated with significantly increased risk of childhood attention-deficit/hyperactivity disorder and autism spectrum disorder. The study concludes that in utero exposure to acetaminophen is associated with increased risk of attention-deficit/hyperactivity disorder and autism spectrum disorder in children and warrant additional investigations. Maternal APAP biomarkers were also associated with increased risk of ADHD diagnosis in offspring.

Carey 2022

The study examined the associations between maternal biomarkers of oxidative stress (OS), measured during pregnancy, and child ASD-related outcomes.⁴²³ The biomarkers of interest included: 8-oxo-deoxyguanine (8-OHdG), an oxidative DNA adduct; Glutathione (GSH), the primary intracellular ROS antioxidant and detoxification mechanism in the body; Glutathione disulfide (GSSG), the oxidized counterpart to GSH and a marker of extra-cellular oxidative stress; the ratio of GSH:GSSG, which acts as an indicator of redox balance; and 3-nitrotyrosine (nitrotyrosine), an amino acid modified by nitrosylation that is formed under oxidative/nitrosative stress.

Outcome definition and determination: ASD-related outcomes were assessed via the Social Responsiveness Scale (SRS), Mullen Scales of Early Learning (MSEL-ELC), and ASD clinical diagnosis at 36 months. The SRS is a questionnaire designed to assess a child's reciprocal social behaviors in social interactions. The MSEL assesses early intellectual development and school readiness that is strongly correlated with intellectual quotient (IQ) scores. ASD diagnosis at 36 months was determined by clinical evaluation, which included the MSEL and the Autism Diagnostic Observation Schedule (ADOS).

Control group: This study did not have a traditional control group as it was a cohort study following pregnancies among women who already had an autistic child.

Study size: In total, 173 participants were eligible based on the outlined criteria however, the total number of participants included in final analyses varied by outcome of interest, from 149 to 164 participants.

Confounding factors or biases and how or if they were controlled: Covariates included in analyses were selected based on a priori knowledge, as well as statistical relevance. The primary models were adjusted for maternal age, race/ethnicity, maternal pre-pregnancy body mass index (BMI), child's sex, and prenatal vitamin use in the first month of pregnancy. Additional covariates considered in sensitivity analyses included pregnancy complications, smoking status, gestational age at DNA/plasma sampling, antioxidant intake, and use of folic acid supplementation in the first month of pregnancy.

Limitations: Reported limitations included limited timing, and familial background risk could change the nature of associations.

⁴²³ Carey et al. Examining associations between prenatal biomarkers of oxidative stress and ASD-related outcomes using quantile regression. *J Autism Dev Disord.* 2022 Jun 9;10.1007/s10803-022-05625-9. doi: 10.1007/s10803-022-05625-9. Epub ahead of print. PMID: 35678944; PMCID: PMC9732143.

Results: Increasing GSH:GSSG was associated with minor increases in SRS scores (50th percentile β : 1.78, 95% CI: 0.67, 3.06). Significant crude ORs for oxidized glutathione and ASD (GSSG, OR 2.27, 95% 1.07, 4.81) were not significant after adjustments (aOR = 2.03, 95% CI 0.89, 4.62).

The conclusion of the study is that there was not a strong relationship between the biomarkers of oxidative stress (OS) measured in maternal samples from mid- to late-pregnancy and child ASD-related outcomes measured at 36 months. However, there was evidence that greater antioxidant balance (higher GSH:GSSG ratio) was inversely associated with risk of ASD and non-typical development according to diagnostic assessments.

Weight of the Evidence for the Association of APAP with ASD

Overall, **these studies provide “some evidence” that APAP use during pregnancy causes developmental toxicity in offspring**, this conclusion is based on study limitations and the reported association between APAP use during pregnancy and ASD outcomes in offspring.

Based on the totality of reviewed studies indicated above and summarized below, the weight of the evidence indicates **there is a moderate association of APAP exposure during pregnancy with ASD or related outcomes in children**. Regarding dose, “therapeutic” dosages are sufficient to cause harm, with higher concentrations being associated with higher risk, aOR = 2.88 (95% CI 1.80-4.66), see Ji 2020. Regarding duration, a longer duration increases risk, with an aHR = 1.23 (95% CI 1.02-1.48) with 2-5 weeks of exposure, see Liew 2016, and a 1.49-2.01 increased risk reported for exposure throughout pregnancy, summarized below. There are multiple studies and lines of evidence that support the association, but some studies did not detect an association. Factors that support and affect the weight of the evidence include:

- The study designs varied from cohort studies, case-control studies, to internet-based surveys, which have different strengths and limitations in terms of validity, reliability, and generalizability.
- The exposure and outcome assessments vary from self-report, medical records, biomarkers, to standardized tests, which have different sources of error, bias, and confounding.
- The control groups vary from children without ASD or related outcomes, children without APAP exposure, to children with other developmental disorders, which have different implications for comparison and interpretation.
- The confounding factors vary from maternal age, education, smoking, BMI, to genetic and environmental factors, which have different effects on the association and may not be fully controlled for in all studies.

The range of odds ratios support the association between APAP exposure during pregnancy or childhood and ASD are reported below, with multiple studies supporting significant positive associations. This means that APAP exposure during pregnancy or childhood increases the risk of ASD or related outcomes in children compared to no exposure. The data from these studies are summarized below:

Study	Exposure	Outcome	Findings
Avella-Garcia 2016	Prenatal APAP exposure	Autism spectrum symptoms in males	1.41 (95% CI 1.01–1.98) IRR
Avella-Garcia 2016	Prenatal APAP exposure	Commission errors in attention function	1.10 (95% CI 1.03–1.17) IRR
Bittker 2018	Postnatal APAP exposure	ASD	1.016 (95% CI 1.003–1.032) aOR
Liew 2016c	Prenatal APAP exposure	ASD	1.19 (95% CI 1.04–1.35) for ever versus never use; 1.39 (95% CI 1.14–1.70) for use during all three trimesters; 1.39 (95% CI 1.14–1.70) for >20 weeks use, aHR
Liew 2016c	Prenatal APAP exposure	infantile autism	1.49 (95% CI 1.07–2.07) for use during all three trimesters; 1.62 (95% CI 1.05–2.51) for >20 weeks use, aHR
Liew 2016c	Prenatal APAP exposure	ASD or infantile autism with HKS	ASD: 1.51 (95% CI 1.19–1.92) for ever versus never use; 1.77 (1.24–2.53) for use during all three trimesters, aHR; 1.51 (95% CI 1.14–2.00), 1.62 (95% CI 1.08–2.45), 1.89 (95% CI 1.19–3.02) for 1-5, 6-20, >20 weeks, respectively Infantile: 2.45 (95% CI 1.32–4.53) for use during all three trimesters; 2.25 (95% CI 1.00–5.07) for >20 weeks use, aHR
Ji 2020	Cord plasma biomarkers	ASD	2.88 (95% CI 1.80–4.66) for the highest concentration group, aOR
Ji 2020	Cord plasma biomarkers	ADHD	3.72 (95% CI 1.70–8.55) for the highest concentration group, aOR
Ji 2020	Cord plasma biomarkers	ASD and ADHD	3.38 (95% CI 1.25–9.85) for the highest concentration group, aOR

Avella-Garcia 2016:

- Prenatal APAP exposure was associated with more autism spectrum symptoms in males (incidence rate ratio (IRR) = 1.41, 95% CI 1.01–1.98) and adverse effects on attention function in both genders (IRR = 1.10, 95% CI 1.03–1.17 for commission errors; β = 0.75, 95% CI 0.13–0.02 for detectability scores).

Bittker 2018:

- Postnatal APAP exposure was associated with ASD (adjusted odds ratio (aOR) = 1.016, 95% CI 1.003–1.032), along with postnatal antibiotic use (aOR = 1.103, 95% CI 1.046–1.168), incidence of ear infection (aOR = 1.137, 95% CI 1.046–1.236), and early weaning (aOR 0.948, 95% CI 0.932–0.965). Oral vitamin D drop exposure was weakly associated with ASD (aOR 1.025, 95% CI 0.995–1.056).

Liew 2016c:

- Prenatal APAP exposure was associated with ASD (adjusted hazard ratio (aHR) = 1.19, 95% CI 1.04–1.35 for ever versus never use; aHR = 1.39, 95% CI 1.14–1.70 for use during all three trimesters; aHR = 1.39, 95% CI 1.14–1.70 for >20 weeks use). Longer duration of use increased the risk of ASD or infantile autism with hyperkinetic symptoms almost twofold (aHR = 1.49, 95% CI 1.07–2.07 for use during all three trimesters; aHR = 1.62, 95% CI 1.05–2.51 for >20 weeks use).
- Prenatal APAP exposure was associated with ASD accompanied by hyperkinetic symptoms (adjusted hazard ratio (aHR) = 1.51, 95% CI 1.19–1.92 for ever versus never use; aHR = 1.77, 95% CI 1.24–2.53 for use during all three trimesters; aHR = 1.89, 95% CI 1.19–3.02 for >20 weeks use). Longer duration of use increased the risk of infantile autism accompanied by hyperkinetic symptoms almost twofold (aRR = 2.45, 95% CI 1.32–4.53 for use during all three trimesters; aRR = 2.25, 95% CI 1.00–5.07 for >20 weeks use).

Ji 2020:

- The study found that the highest concentrations of APAP in cord blood were associated with ASD (aOR = 2.88, 95% CI 1.80–4.66), ADHD (aOR = 3.72, 95% CI 1.70–8.55), and ASD and ADHD (aOR = 3.38, 95% CI 1.25–9.85). Other developmental disorders were not associated with APAP concentrations (aOR = 0.86, 95% CI 0.56–1.31).

Carey 2022:

- Increasing GSH:GSSG was associated with minor increases in SRS scores (50th percentile β : 1.78, 95% CI: 0.67, 3.06). Significant crude ORs for oxidized glutathione and ASD (GSSG, OR 2.27, 95% 1.07, 4.81) were not significant after adjustments (aOR = 2.03, 95% CI 0.89, 4.62).

B. Studies Examining the Association of APAP with ADHD

Anand 2021

This study used the Boston Birth Cohort (BBC), a prospective birth cohort study of 3165 mother-child pairs followed at the Boston Medical Center (BMC) from 1998 to 2018.⁴²⁴

Outcome definition and determination: Childhood neurodevelopmental diagnoses were categorized based on clinician documentation of primary and secondary diagnoses in electronic medical records up to June 2018. The ADHD-only category included children with only ADHD-related codes (ICD-9 codes 314.0–314.9; ICD-10 codes F90.0–F90.9) and excluded children with autism spectrum disorder (ASD) diagnoses (ICD-9 codes 299.0–299.91; ICD-10 codes F84.0–F84.9) or other mental, behavioral, and neurodevelopmental disorders (ICD-9 codes 290–319; ICD-10 codes F01–F99).

⁴²⁴ Anand et al. Perinatal Acetaminophen Exposure and Childhood Attention-Deficit/Hyperactivity Disorder (ADHD): Exploring the Role of Umbilical Cord Plasma Metabolites in Oxidative Stress Pathways. *Brain Sci.* 2021 Sep 30;11(10):1302. doi: 10.3390/brainsci11101302. PMID: 34679367; PMCID: PMC8533963.

Control group: The neurotypical development category included children without ADHD, ASD, or other developmental disability diagnoses.

Study size: The source population of 3165 mother-child dyads included 433 (13.7%) participants with a childhood diagnosis of ADHD only and 1444 (45.6%) with neurotypical development. Of these, 965 participants had cord plasma analyte data when excluding siblings, of which 248 (25.7%) had childhood ADHD and 320 (33.2%) had neurotypical development.

Confounding factors or biases and how or if they were controlled: The study included several covariates drawing upon previously published analyses of the BBC such as maternal age at delivery, parity, maternal race/ethnicity, maternal education level, maternal BMI, stress during pregnancy, smoking during pregnancy, alcohol use before or during pregnancy, marital status, child sex, delivery type, preterm birth, low birthweight, stress during pregnancy, and maternal fever during pregnancy.

Limitations: The study examined cord blood, removing recall bias, but limiting temporal exposure to late gestation. Other limitations include unmeasured, residual confounding, and genetics.

Results: Cord unmetabolized APAP >50th percentile was associated with higher odds of ADHD diagnosis (aOR: 2.10, 95% CI 1.43, 3.11). In adjusted regressions, increasing levels of cord methionine (aOR: 1.43, 95% CI 1.17, 1.77), serine (aOR: 1.38, 95% CI 1.14, 1.68), glycine (aOR: 1.38, 95% CI 1.14, 1.68), and 8-hydroxy-deoxyguanosine (aOR: 1.24, 95% CI 1.01, 1.52) were associated with higher odds of ADHD.

Overall, the study concludes APAP was associated with increased risk of ADHD. The amino acids involved in producing the antioxidant glutathione, including methionine, serine, and glycine, were also associated with increased odds of ADHD. Additionally, DNA oxidation (8-hydroxy-deoxyguanosine), a marker of oxidative damage, was also linked to ADHD.

Baker 2020

This was a prospective cohort study.⁴²⁵

Information used to determine exposure: Prenatal APAP exposure was measured in meconium.

Outcome definition and determination: The outcome was ADHD in children aged 6 to 7 years, as determined by the Conners' Parent Rating Scale-Revised: Long Form.

Control group: The control group consisted of children who were not exposed to acetaminophen prenatally.

Study size: The study included 394 eligible children with 345 meconium samples collected at birth.

Confounding factors or biases and how or if they were controlled: The study controlled for several potential confounding factors, including maternal age, education, smoking during pregnancy, alcohol consumption during pregnancy, and child sex.

⁴²⁵ Baker et al. Association of Prenatal Acetaminophen Exposure Measured in Meconium With Risk of Attention-Deficit/Hyperactivity Disorder Mediated by Frontoparietal Network Brain Connectivity. *JAMA Pediatr.* 2020 Nov 1;174(11):1073-1081. doi: 10.1001/jamapediatrics.2020.3080. PMID: 32986124; PMCID: PMC7522774.

Limitations: The study had several limitations, including the potential for residual confounding and the possibility that meconium may not accurately reflect self-reported prenatal acetaminophen exposure. Meconium can identify drug exposure in the 2nd and 3rd trimester and reveal the relative concentration compared to others in the cohort, but cannot by itself identify exact dose or duration.

Results: Detection of APAP in meconium was associated with increased odds of ADHD (OR = 2.43, 95% CI, 1.41-4.21). A dose-response association was detected; each doubling of exposure increased the odds of ADHD by 10% (OR = 1.10, 95% CI 1.02-1.19).

Overall, the study found that children exposed to acetaminophen prenatally were at increased risk of ADHD at ages 6 to 7 years. Prenatal acetaminophen exposure was also associated with increased negative connectivity between the left prefrontal cortex and the right precentral/frontal gyrus, which mediated the association of acetaminophen with hyperactivity.

Chen 2019

The study is a nationwide case-control study that investigated the association between prenatal exposure to acetaminophen and the risk of attention-deficit/hyperactivity disorder (ADHD) in children.⁴²⁶

Information used to determine exposure: The exposure of interest was prenatal exposure to acetaminophen, which was assessed using data from Taiwan's National Health Insurance Research Database.

Outcome definition and determination: The outcome of interest was a diagnosis of ADHD in children, which was determined using International Classification of Diseases, Ninth Revision (ICD-9) codes from national registers.

Control group: The control group consisted of children without ADHD who were matched to cases by age and sex.

Study size: The study included 950 mother children pairs with ADHD and 3,800 control pairs.

Confounding factors or biases and how or if they were controlled: The study controlled for several potential confounding factors, including maternal age at delivery, parity, maternal smoking during pregnancy, maternal pre-pregnancy body mass index (BMI), maternal education, maternal psychiatric history, and family income. The authors used conditional logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

Limitations: Proposed limitations included underestimated prevalence of ADHD and potential misclassification of controls if some had ADHD, which would bias towards the null hypothesis. Self-administration of APAP may have occurred if it was purchased over-the-counter, as use was based on prescriptions. Potential interactions with other medications, bias confounding. There was also potential misclassification of mothers, because no mothers were diagnosed with ADHD or substance use disorders.

Results: APAP exposure in the second trimester (OR = 1.19, 95% CI 1.00-1.40), first and second trimester (OR = 1.28, 95% CI 1.00-1.64), or any trimester (OR = 1.20, 95% CI 1.01-1.42) was associated with an increased risk of ADHD in offspring. Associations remained after excluding gestational infections and

⁴²⁶ Chen et al. Prenatal Exposure to Acetaminophen and the Risk of Attention-Deficit/Hyperactivity Disorder: A Nationwide Study in Taiwan. *J Clin Psychiatry*. 2019 Sep 10;80(5):18m12612. doi: 10.4088/JCP.18m12612. PMID: 31509360.

maternal health disorders (OR = 1.33, 95% CI 1.04-1.69)(OR = 1.68, 95% CI 1.18-2.40)(OR = 1.40, 95% CI 1.14-1.73), respectively.

Overall, the study concluded that prenatal exposure to APAP is associated with an increased risk of ADHD in children.

Ji 2018

This is a prospective cohort study design using samples selected from the Boston Birth Cohort.⁴²⁷

Information used to determine exposure: Information about APAP exposure was based on maternal biomarkers of APAP intake measured in plasma samples obtained within 1–3 days postpartum.

Outcome definition and determination: The outcome of interest was ADHD diagnosis in offspring. ADHD diagnoses were obtained from electronic medical record review.

Control group: The control group consisted of children without ADHD diagnosis.

Study size: The study included a total of 1180 children, including 188 with ADHD diagnosis.

Confounding factors or biases and how or if they were controlled: Potential covariates such as maternal age, race/ethnicity, education, marital status, pre-pregnancy body mass index, smoking during pregnancy, alcohol use during pregnancy, and child sex were included in the statistical model.

Limitations: The study only measured maternal APAP metabolite levels once, shortly after childbirth, which reflects recent use but cannot provide detailed information on dosage and usage patterns during specific time periods. The measurement method did not include acetaminophen sulfate, a significant metabolite, but estimates of total APAP burden were derived from other measured metabolites. Potential confounding by familial factors and unmeasured or unknown confounders, despite adjustments and propensity score analyses. The study sample predominantly consisted of urban low-income minority populations, limiting the generalizability of the results to all pregnant women in the U.S.

Results: Below median and above median levels of maternal acetaminophen burden were associated with a 58% and 88% increase in the odds of ADHD diagnosis, respectively (Model 6: OR for below median = 1.58, 95% CI 1.02-2.46; OR for above median 1.88, 95% CI 1.18, 3.00). However, there were no significant associations found between maternal plasma levels of acetaminophen metabolites and the risks of ASD diagnosis and other DD diagnoses.

The study concludes that maternal plasma biomarkers of APAP use shortly after delivery were associated specifically with an increased risk of ADHD diagnosis in offspring. No associations were found with other developmental disorders. These findings remained significant even after adjusting for known confounders and indications for APAP use.

⁴²⁷ Ji et al. Maternal Biomarkers of Acetaminophen Use and Offspring Attention Deficit Hyperactivity Disorder. *Brain Sci.* 2018 Jul 3;8(7):127. doi: 10.3390/brainsci8070127. PMID: 29970852; PMCID: PMC6071105.